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A MARGARINE-SUPPLEMENTED DIET ALONE AND IN COMBINATION WITH CHAMOMILE DECOCTION DECREASES FOOD INTAKE BUT HAS A MILD EFFECT ON BODY MASS IN MICE

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Abstract: The Western diet is one of the most popular eating styles in recent years. It is based on foods high in fat, sugar and salt. It is known that excessive consumption of this type of food causes the development of metabolic syndrome (MetS), characterized by obesity, insulin resistance, high cholesterol and blood glucose levels, and high blood pressure. The aim of this study was to test whether diet enriched with margarine, as the main component of Western diet foods, to be able to induce obesity in experimental mice and whether chamomile water decoction (CWD) was able to mitigate effects of margarine-containing diet. The latter, as can be seen from previous studies, has a high potential to mitigate MetS. We also decided to test whether a diet with margarine and CWD would have the same effect on young mice of both sexes. For the experiment, one-month-old male and female mice of the C57Bl/6J line were used. The mice were divided into three groups, six in each. The first group (control) consumed basic food and water during the entire experiment. The second group consumed basic food and 70% fat margarine, added in excess in a separate dish, and drank water. The third group consumed basic food and margarine (70% fat) and drank CWD diluted in drinking water. The experiment lasted four months. During the experiment, the animals' body mass, food and water consumption were monitored with calculation of body mass index (BMI) and Lee obesity index at the end of feeding period. The results showed that the addition of margarine to the basic diet caused an increase in body mass in females, but not in males, but did not changed BMI or in the Lee obesity index in both sexes. The addition of CWD to the margarine-containing diet caused a downward trend in body mass gain and reduced the Lee obesity index in both sexes. Mice, which were fed margarine-containing diet, showed by 15% and 30% by lower total food intake in males and females, respectively, as compared with the control group. At the same time, no difference in a number of calories was observed between control and margarine-fed groups. On the margarine-supplemented diet, males consumed an average of 70% basal food and 30% margarine, while females' diet consisted of approximately 50% basal food and 50% margarine In addition, water consumption decreased in mice fed margarine alone and in the CWD background.. Consumption of CWD with a margarine diet increased food intake in males to the level of the control group, but had no similar effect in females. As in margarine-fed mice, mice fed margarine and CWD showed a downward trend in water consumption. It can be concluded that the mice were not obese.

Keywords: body mass, chamomile decoction, food consumption, margarine, metabolic syndrome, mice.

1. INTRODUCTION

The combination of an unhealthy diet and a sedentary lifestyle leads to metabolic disorders that increase the risk of developing obesity, cardiovascular disease and type II diabetes (Bayliak, 2020; Hurza et al., 2021). These diagnoses are an integral part of the metabolic syndrome (MetS), a set of pathological conditions characterized by abdominal obesity, insulin resistance, hypertension and hyperlipidemia (Bayliak, 2020; Hurza et al., 2021; Vatashchuk et al., 2022).

It is established that MetS develops as a result of excessive consumption of foods containing high levels of fat and carbohydrates (Santos-Marcos et al, 2019; Hurza et al., 2021). Many studies conducted on mice and rats confirm the negative effects of high-calorie diets on the health status

(Avtanski et al., 2019; Vatashchuk et al., 2022). In particular, diets containing industrially produced trans-unsaturated fatty acids (TUFAs) increase the risk of cardiovascular disease and inflammation (Calder et al, 2011; Valenzuela at al., 2019). The main product containing TUFAs is margarine, which is produced as a result of industrial hydrogenation of vegetable oils (Emken., 1984; Valenzuela at al., 2019). Previous studies in rodents have shown that long-term consumption of margarine or other TUFAs leads to the development of metabolic disorders of varying degrees, including obesity (Satta et al., 2018; Zhu et al., 2019 b), non-alcoholic fatty liver disease (Oteng et al., 2019), and atherosclerosis (Monguchi et al., 2017).

Traditionally, foods containing industrially produced TUFAs are part of the Western diet (Ganguly & Pierce, 2015; Valenzuela at al., 2019). This style of eating has become very popular in recent years and involves the consumption of bakery products, chips, spicy, fried foods, etc., as well as foods high in sugar, salt and fat (Stevenson at al., 2020).

Given that margarine is a part of the Western diet and can cause metabolic disorders, we decided to check whether MetS alone is able to induce obesity in model animals. Also, given that the previous experiments studied the effect of margarine on only one sex of animals (males), we decided to test whether the consumption of this product had the same effect on male and female experimental mice. For the experiment, we have chosen 70% fat margarine, since previous research showed that diets with a fat content of more than 40% are most often used to develop obesity in experimental animals (Avtanski et al., 2019; Wang et al., 2020; Vatashchuk et al., 2022).

Chamomile (*Matricaria chamomilla* L.) is a widespread medicinal plant (Bayliak et al., 2021; El Mihyaoui et al., 2022). It is often used in traditional Ukrainian medicine as a treatment for digestion, gynecological and skin problems, eye and mouth infections and is also used as a sedative and analgesic remedy (Singh et al., 2011; Bayliak et al., 2021). It is assumed that the biologically active substances present in chamomile flower, in particular phenolic compounds, provide the pharmacological properties of this plant (Singh et al., 2011). Apigenin is one of the flavanoids with the highest concentration in chamomile flowers (about 17%) and is used for standardization of chamomile preparations (McKay & Blumberg, 2006; Miguel et al., 2015). There are studies that show that addition of apigenin to a high-calorie diet leads to a reduction in body mass, glucose levels, plasma triacylglycerols and other indicators of obesity (Yang et al., 2018; Lv et al., 2019; Qiao et al., 2022).

To prevent the development of metabolic changes in mice as a result of margarine consumption that could occur, we used a water decoction of chamomile flowers (CWD). Our choice on the decoction of chamomile flowers is justified by the fact that it is chamomile tea - the main form of consumption of chamomile preparations in everyday life and for medical reasons. It is also known from previous studies that consumption of CWD in the context of diets high in fat or carbohydrates shows antioxidant effects and the ability to reduce the manifestations of MetS (Jabri et al., 2017; Bayliak et al., 2021; Jabri et al., 2022). In this study, we compared a sex-dependent intensity of food consumption and body mass gain in mice that consumed a standard rodent chow only, a standard diet with addition of margarine in unlimited amounts (a margarine diet), and a margarine diet with CWD instead drinking water.

2. MATERIALS AND METHODS

2.1. Design of the experiment. C57Bl/6J mice of both sexes were used for the study. Until mice reached one month of age, they were on a basic diet (a standard food for laboratory animals of "Rezon-1" PE, Kyiv, Ukraine). The basic diet consisted of 21.8% protein, 4.8% fat, 69.1% carbohydrates and 3.9% fiber. After the mice reached the required age (1 month), they were randomly switched to experimental diets. Each group consisted of six animals (separately males and females) with three mice per cage (two cages per one group). The first group (control) continued to consume the basic diet. The second group (margarine), in addition to the basic diet, was given margarine of 70% fat content (Soniachnyi Shchedryk, TM Olkom). Margarine was given in excess in separate dishes and mice had choice to eat basic food or margarine. Portions of margarine were replaced every two days. The third group of mice (margarine + CWD) consumed a standard food and margarine, but instead of water, they were given an aqueous decoction of chamomile flowers mixed with the drinking water in a 1:1 ratio as described below. The decoction was changed every day to avoid bacterial contamination. All groups had unlimited access to water (or CWD) and food. A standard food was refreshed once a week. The mice were kept on the experimental diets for 4 months. All mice were kept in a 12-hour light/dark cycle (light period from 6 h to 18 h) at an air temperature of 22 ± 2°C and humidity of 50-60%.

2.2. Preparation of a water decoction of chamomile flowers. The decoction was prepared using a pharmaceutical preparation from the "Viola" pharmaceutical factory. Dried chamomile flowers were ground in a mortar and poured with water in a ratio of 1:30. Then the mixture was boiled, and after boiling, it was cooked for another 5 minutes. The resulting broth was cooled, filtered and made up to the original volume with distilled water. This chamomile decoction was frozen and stored in a freezer at -20°C. Before use for mice, the decoction was thawed at room temperature and diluted 1:1 with drinking water (Sebai et al, 2015).

2.3. Determination of physiological parameters. The body mass, the amounts of food consumed, water or chamomile decoction drunk were monitored during the entire experiment. The experimental animals were weighed weekly using electronic scales. The amount of consumed food was also measured once a week. The amount of margarine, chamomile water decoction and water consumed by the animals were measured at their refreshening - every 2 days, once a day and once a week, respectively. The measurements were taken between 9-10 a.m. The body length of the mice was determined before sacrifice by measuring the distance from the tip of the animal's nose to the base of the tail from the abdominal side. The length was measured in centimeters using a ruler. The body mass measured before sacrifice was used to calculate the body mass index (BMI) and the Lee obesity index (Cortellini et al., 2019; Zhu et al., 2019 a).

2.4. Statistical analysis of the results. Statistical analyses were performed using Microsoft Excel 2010 and GraphPad Prism 6 software. One-way ANOVA followed by the Duncan's test was used to determine the statistical difference between groups. The difference between groups was considered significant when the value of $P \leq 0.05$. Comparisons were made between all groups participating in the experiment, as well as between the sexes.

3. Results and Discussion

Fig. 1 shows the changes in body mass of C57Bl/6J mice consumed the experimental diets for 16 weeks. At the end of the experiment, the control groups of mice weighed 21.4±0.9 g for males and 18.7±1.3 g for females. Generally, mice showed slightly lower body masses than mice of the same strain and age in other studies (Bachmanov et al. (Bachmanov et al., 2002; Yang et al., 2014). In particular, in the study of Bachmanov et al. C57Bl/6J mice aged between 65 and 74 days had a body mass of about 25 g (Bachmanov et al., 2002). At the same time, there are studies in which the body mass of mice was close to our results (Rainwater and Güler, 2022). Perhaps, the observed differences can be due to some differences in the food composition, because even a standard rodent chow is produced by different manufacturers. Margarine-fed groups showed a tendency to gain a higher body mass than the control ones (Fig. 1A, B). Due to intragroup variability, only margarine-fed males has significantly higher body mass than the control mice at the end of the experiment (Fig. 1C). Male mice consumed margarine had 23% higher body mass than the control ones at the end of the experiment. The addition of CWD to the margarine diet resulted in a 12% lower male body mass compared to the margarine group. No significant effect of margarine alone and CWD in combination with margarine was found on body mass of females as compared with the control group.

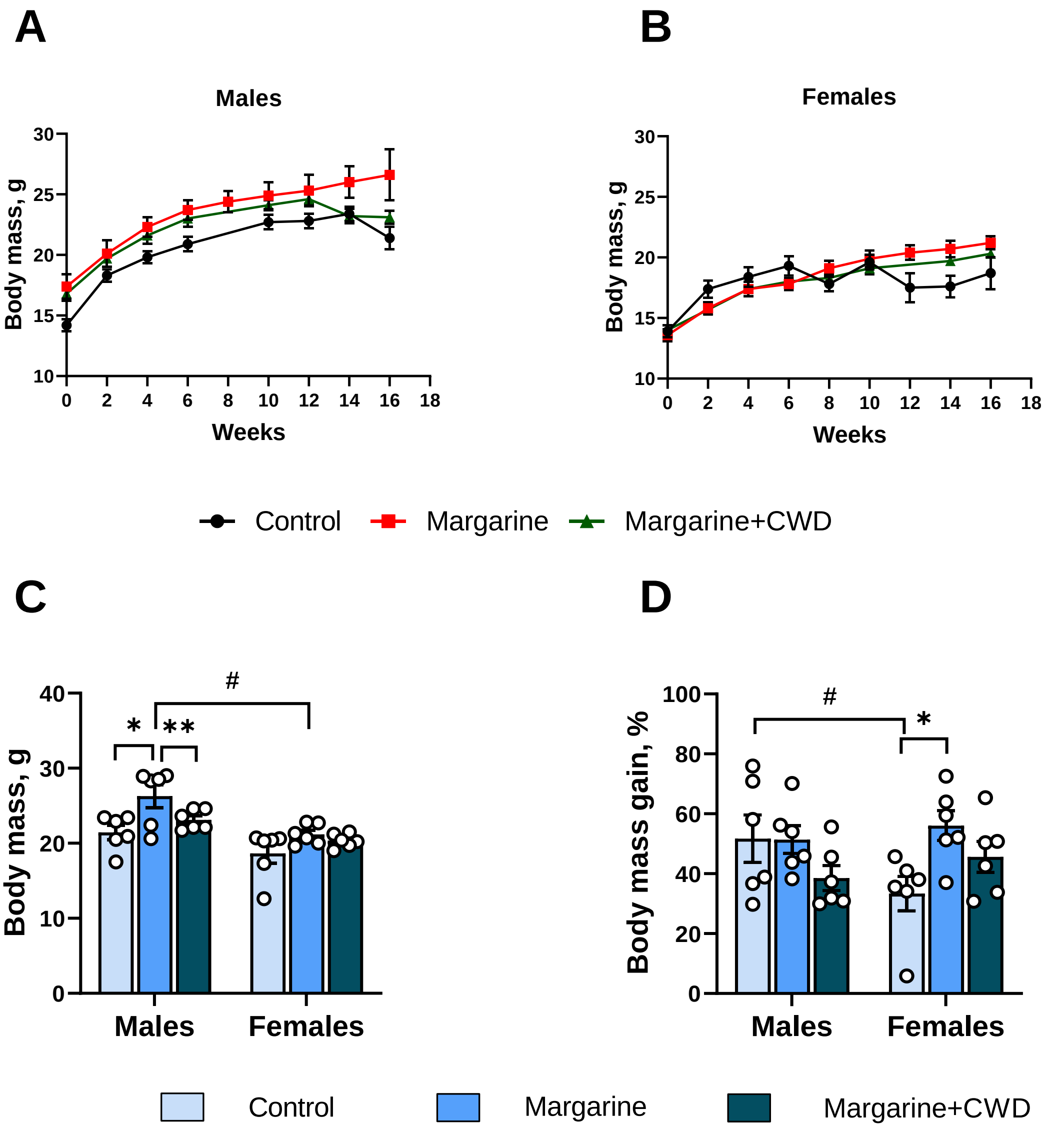

*Figure 1. Body mass of mice fed the basic food (control group), basic food with margarine (margarine group) and basic food with margarine and chamomile water decoction (margarine + CWD group) in drinking water. Body mass dynamics of males (A) and females (B); .body mass at the end of the experiment (C), and body mass gain (D). *Significantly different from the corresponding control group with P ≤ 0.05. ** Significantly different from the corresponding margarine group with P ≤ 0.05. #Significantly different between sexes with P ≤ 0.05.*

It should be noted that due to random allocation of mice to groups at the start of the experiment, mice had a little different initial body masses in the groups (Fig. 1 A, B). Since initial body mass might determine the final body mass, we calculated body mass gain in percentages relatively to the initial body mass (Fig. 1 D). Thus, we found that consumption of margarine alone did not affect body mass gain in males but caused a 40% increase in body mass in females compared to the control group. The addition of CWD to the margarine diet caused a tendency to slow dawn body mass gain in both sexes. Research by Zhou et al., 2020 showed that eating a high-fat diet caused an increase in body mass in male mice. Other research showed that in rats, the consumption of trans-unsaturated fatty acids (Zhu et al., 2019 b) and margarine (Satta et al., 2018) also led to an increase in body mass.

In our study, we observed an increase in body weight gain only in females when consuming margarine. This is contrary to the study of Fan et al. (2020), in which C57Bl/6J males of approximately the same age gained body mass by two times faster when margarine was added to the diet. Studies demonstrated that it takes at least 10 weeks for rats to receive a clear difference in the increase in body mass between animals fed a standard and a high-calorie diet (Noeman et al., 2011). It is likely that this period is longer for mice. A study by Jabri et al (2022) showed that the addition of CWD to a high-fat diet caused a decrease in body mass in male rats. In this study, we observed a similar tendency to lower body mass in both sexes but significant difference was not found. It is possible that in our case, the time spent by the mice on the experimental diets was insufficient to detect a statistical difference.

Next, we calculated obesity indexes in mice fed experimental diet. As obesity index we used a standard body mass index (BMI) and the Lee obesity index, specific for rodents. The indexes were calculated on the last day of the experiment, before the animals were sacrificed. Figures 2 A and 2 B demonstrate that there is no statistical difference in these parameters in mice that consumed only margarine. However, the addition of CWD to the margarine diet reduced the Lee obesity index in both males and females by 10% and 17% compared to the control and by 10% and 19% compared to the margarine group. This indicates that mice fed a diet with margarine were not obese despite their higher body mass was higher than in the control ones.

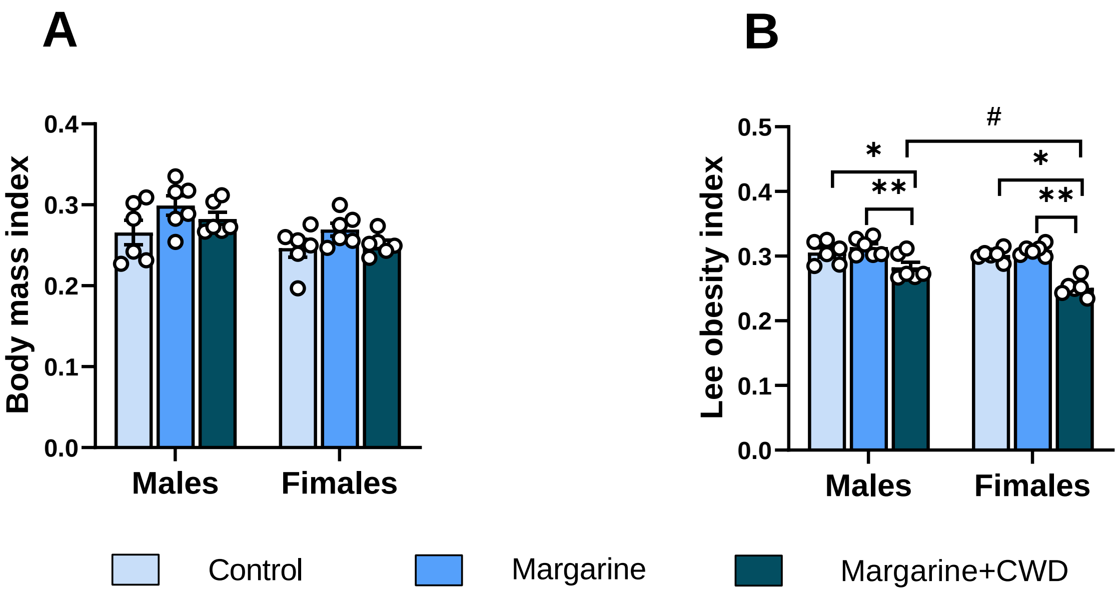

Figure 2. Body mass index(A) and Lee obesity index (B) in mice fed experimental diet. Another information is the as in Figure 1.

In our experiment, C57Bl/6J mice also consumed less a basic food (Fig. 3 A, B) compared to the values typical for this line in other studies (Bachmanov et al., 2002). Total food intake decreased in mice when margarine was added to the basic diet, especially in female mice (Fig. 3 A, B). Male and female mice, which were fed margarine-containing diet, demonstrated by 15% and 30% lower total food intake, respectively, compared to the control group. In the study by Satta et al. (2018), where rats consumed margarine every day, the amount of food consumed did not differ between control and margarine fed groups. Other studies demonstrated decrease in total amount of food consumed when were fed with high-calorie diet. (Nakandakari et al., 2019; Muthuramalingam et al., 2020; Casimiro et al., 2021). This is consistent with our results. On the margarine-supplemented diet, males consumed an average of 70% basal food and 30% margarine, while females' diet consisted of approximately 50% basal food and 50% margarine

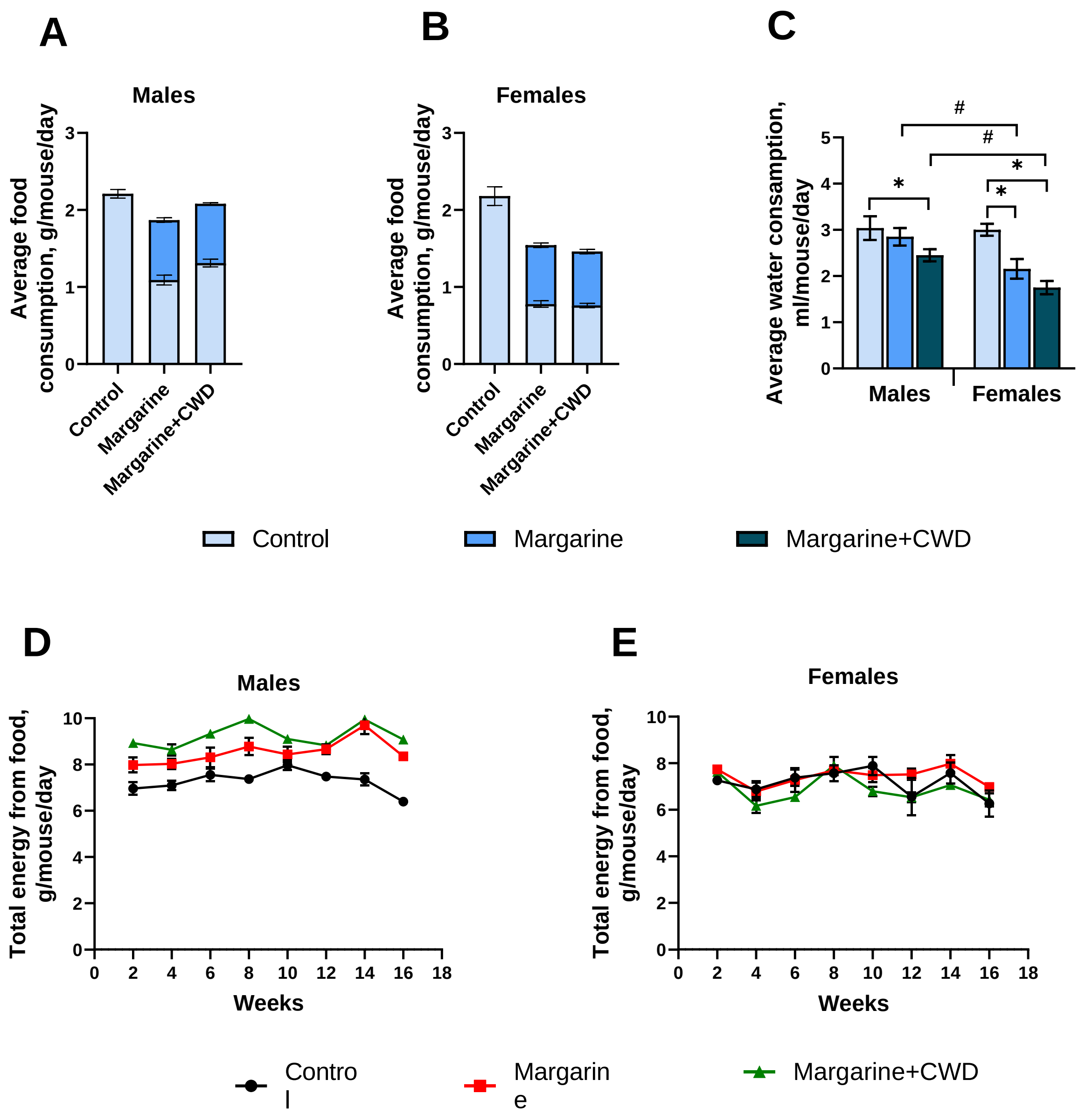

Figure 3. Food and water/CWD consumption in mice fed experimental diets, *Average food consumption by males (A) and females (B), average water consumption (C), dynamics of total energy obtained from food in males (D) and females (E). Another information is the as in Figure 1.*

We also showed that adding margarine to the diet reduced water consumption in females by 28% (Fig. 3 C). Replacing water with CWD led to a 19% reduction in water consumption in males and a 42% reduction in females compared to the control group. The water consumption of females on the margarine diet was 25% lower than that of males on the same diet. The consumption of CWD by females was 29% lower than that of males. However, previous study showed increased water intake in mice on a high-calorie diet (Hou et al., 2010). Recent studies have confirmed a reduction in food and water intake with a high-fat diet (Li et al., 2021; Su et al., 2022). Yang et al. (2018) reports that adding apigenin (the main component of chamomile flowers) to drinking water did not change the intensity of water intake compared to the control group.

Male mice, that consumed margarine, received more energy from food compared to the control group (Fig. 3 D). These results are in line with other studies (Liu et al., 2019; Zhao et al., 2021) where male mice got more energy when consumed a high-calorie diet. Males on the diet containing margarine in combination with CWD also received more daily calories compared to the control group of mice. No such difference was observed in females. This can be explained by the fact that males consumed more food than females on the margarine and margarine + CWD diets (Fig. 3 A, B).

4. CONCLUSION

This study demonstrates that consumption of margarine alone did not cause the development of visible signs of obesity in both sexes of mice. This is supported by the absence of significant changes in BMI and Lee obesity index. However, body mass gain in females consuming margarine was higher than in that on the basic diet. Consumption of chamomile water decoction, CWD, in combination with a margarine diet also did not affect BMI but reduced the Lee obesity index.. However, CWD caused a tendency to decrease body mass gain in both sexes. The addition of margarine to the basic diet reduced the average food and water intake of both female and male mice. The consumption of CWD with the margarine diet alsoreduce water consumption in both sexes and caused an increase in the average food intake to the values of the control group in males but not in on females. Thus, the margarine-supplemented diet reduces food and water intake in both sexes of mice and does not affect body mass gain in males. The addition of chamomile decoction to margarine diet causes a downward trend in body weight in both sexes, that suggest a possible protective effect of this plant against overweight. Detailed biochemical effects of and molecular mechanisms of margarine and CWD will be studied in our next experiments.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical statements: All mouse protocols were approved by the Animal Experimental Committee of Vasyl Stefanyk Precarpathian National University (Ukraine) and were conducted in accordance with the European Union for the protection of animals used for scientific purposes of 22 September 2010 (2010/63/EU).

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Вікторія Гурза, Мирослава Ватащук, Марія Байляк. *Дієта з додаванням маргарину окремо та в поєднанні з відваром ромашки зменшує споживання їжі, але має помірний вплив на масу тіла мишей.* *Журнал Прикарпатського університету імені Василя Стефаника*, 10 (2023), 45-55.

Західна дієта - один з найпопулярніших стилів харчування в останні роки. В її основі лежить їжа з високим вмістом жирів, цукру та солі. Відомо, що надмірне споживання такої їжі спричиняє розвиток метаболічного синдрому, який характеризується ожирінням, інсулінорезистентністю, високим рівнем холестерину та глюкози в крові, а також підвищеним кров'яним тиском. Метою цього дослідження було перевірити, чи може дієта, збагачена маргарином, як основним компонентом продуктів Західної дієти, викликати ожиріння у піддослідних мишей, і чи здатен водний відвар з квітів ромашки пом'якшити наслідки маргариновмісної дієти. Останній, як видно з попередніх досліджень, має високий потенціал для пом'якшення метаболічного синдрому. Ми також вирішили перевірити, чи матиме однаковий вплив на молодих мишей обох статей дієта з маргарином та відваром з квітів ромашки. Для експерименту використовували одномісячних самців і самок мишей лінії C57Bl/6J. Мишей розділили на три групи, по шість у кожній. Перша група (контрольна) протягом усього експерименту споживала базовий корм і воду. Друга група споживала базовий корм і маргарин 70% жирності, який додавали в надлишку в окремий посуд, і пила воду. Третя група споживала базову їжу та маргарин (70% жирності), а також пила розведений у питній воді (1:1) водний відвар з квітів ромашки. Експеримент тривав чотири місяці. Під час експерименту контролювали масу тіла тварин, споживання їжі та води з розрахунком індексу маси тіла (ІМТ) та індексу ожиріння Лі в кінці періоду годування. Результати показали, що додавання маргарину до основного раціону викликало збільшення маси тіла у самок, але не у самців, але не змінило ІМТ або індекс ожиріння Лі в обох статей.

Додавання водного відвару з квітів ромашки до маргариновмісної дієти викликало тенденцію до зниження приросту маси тіла та знизило індекс ожиріння Лі в обох статей. Миші, які отримували маргариновий раціон, показали на 15% і 30% менше загальне споживання їжі у самців і самок, відповідно, порівняно з контрольною групою. У той же час, різниці в кількості калорій, між контрольною групою та групою, що отримувала маргарин, не спостерігалося. На дієті з додаванням маргарину самці споживали в середньому 70% основної їжі та 30% маргарину, тоді як раціон самок складався приблизно з 50% основної їжі і 50% маргарину. Крім того, миші, яких годували одним маргарином та маргарином на фоні відвару ромашки, споживали менше води. Споживання відвару з квітів ромашки з маргарином збільшило споживання їжі у самців до рівня контрольної групи, але не мало подібного ефекту у самок. Як і у мишей, яких годували маргарином, у мишей, яких годували маргарином і водним відваром ромашки, спостерігалася тенденція до зниження споживання води. Можна зробити висновок, що маргарин-вмісна їжа не призводила до видимих ознак ожиріння у мишей.

Ключові слова: маса тіла, відвар з квітів ромашки, споживання їжі, маргарин, метаболічний синдром, миші.